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HYMENOLEPIS ROBERTRAUSCHI N. SP. FROM GRASSHOPPER MICE *ONYCHOMYS* SPP. IN NEW MEXICO AND NEBRASKA, U.S.A.

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ABSTRACT

From 1989 through 1998, a total of 358 grasshopper mice were collected and examined for helminth and protistan parasites from several habitat types on the Sevilleta Long Term Ecological Research (LTER) site in New Mexico, U.S.A. Of these, 205 individuals were identified as *Onychomys leucogaster* (Wied-Neuwied 1841) and 153 individuals were classified as *O. arenicola* Mearns 1896. Many individuals of *Onychomys* were infected with a new species of Hymenolepididae (*Hymenolepis robertrauschi*), which is herein described, illustrated, and compared with all species of Nearctic *Hymenolepis* s. str. *Hymenolepis robertrauschi* was found in 26% of the individuals of *O. arenicola* and 18% of the individuals of *O. leucogaster*, giving an overall prevalence of 22% in the individuals of *Onychomys* from New Mexico. The intensity of *H. robertrauschi* in *Onychomys* ranged from one to 33 individual cestodes. The number of individuals of *H. robertrauschi* found in each individual of *Onychomys* examined was generally between one and five; only seven of the 79 infected *Onychomys* harbored more than five individual cestodes. In Nebraska, during August 2012 and July-August 2013, 14 specimens of *O. leucogaster* were examined for helminths; *H. robertrauschi* was found in four of those individuals.

Key words: Cedar Point Biological Station, Cestoda, grasshopper mouse, *Hymenolepis robertrauschi*, Mammalia, Nebraska, New Mexico, *Onychomys arenicola*, *Onychomys leucogaster*, Rodentia, Sevilleta Long Term Ecological Research site

INTRODUCTION

Grasshopper mice of the genus *Onychomys* Baird 1857 (Rodentia: Cricetidae) have been little studied relative to their parasite fauna, with only a few papers being published that discuss their helminth and protistan parasites (Cuckler 1939; Doran 1954; Grundmann 1958; Grundmann and Fransden 1960; O'Farrell 1975; Pfaffenberger et al. 1985; Hnida et al. 1998).

As part of a larger study on the parasite fauna of mammals of the Sevilleta Long Term Ecological Research (LTER) site in New Mexico, from 1989 through 1998, grasshopper mice were collected from several habitats on the LTER site and examined for helminth and protistan parasites. Individuals of two species, *Onychomys leucogaster* (Wied-Neuwied 1841) and

Onychomys arenicola Mearns 1896, were surveyed for parasites. Later, in 2012 and 2013, as part of a field parasitology course conducted at the Cedar Point Biological Station, University of Nebraska - Lincoln, in western Nebraska, U.S.A., several individuals of *O. leucogaster* were collected from grassland habitats in

the south-central sandhills of Nebraska (Keeler 2012). Comparison of parasites from specimens collected from both species of *Onychomys* from both Nebraska and New Mexico revealed an undescribed species of *Hymenolepis* Weinland 1858; the cestodes found during these studies are described.

METHODS

The material from both New Mexico and Nebraska and used for the following description was collected between May 1989 and August 2013. From the Rio Grande Valley of New Mexico, grasshopper mice were captured from two major habitat types and four specific localities across the Sevilleta LTER site. One hundred fifty-three specimens of *O. arenicola* and 205 specimens of *O. leucogaster* were collected from these localities; all specimens referred to herein have been deposited in the Mammal Division of the Museum of Southwestern Biology, University of New Mexico.

In western Nebraska, during July and August, 2012 and 2013, 14 individuals of *O. leucogaster* were examined, including 11 specimens of *O. leucogaster* collected from sandhills habitat on the Arapaho Prairie (see Keeler et al. 2012) (lat. 41°28'55.9"N, long. 101°50'57.8"W), and three were collected from grassland semidesert habitat at Cedar Point Biological Station (lat. 41°11'35.9"N, long. 101°38'51.0"W). All specimens of *O. leucogaster* that we collected in Nebraska are deposited in the Zoology Division of the University of Nebraska State Museum.

In all cases, specimens of *Onychomys* were trapped using Sherman™ live-traps baited with rolled oats. Necropsies were performed on individual mice as soon as possible after collection to minimize postmortem changes in the parasites (Gardner and Jiménez-Ruiz 2009). For the specimens from New Mexico, maximum time to necropsy following death of host was usually less than 15 minutes; for specimens examined from Nebraska, maximum time to necropsy was always less than 5 minutes. The contents of each organ of the gastrointestinal tract were examined separately for the presence of helminths with a dissecting microscope.

After necropsy, all cestodes found were transferred to distilled water for a minimum of 20 minutes

to allow the strobilae to relax and they were subsequently fixed and stored in hot 10% (v/v) aqueous formalin solution. In the laboratory, specimens were stained with Semichon's acetic carmine, dehydrated in ethanol, cleared in terpineol and xylene, and mounted permanently in either Canada balsam or damar gum. From specimens collected in Nebraska, ten posterior segments were preserved in 98% ethanol and frozen at -85° C in the Parasite Genomic Research Facility for future investigations using molecules.

The life-cycle of the cestodes found in Nebraska was investigated using beetles [*Tenebrio molitor* (Linnaeus)] as experimental intermediate hosts. General methods for study of the life-cycle followed those of Gardner and Schmidt (1988); however, with respect only to those forms collected in Nebraska, six adult specimens of *T. molitor* (Linnaeus) were each fed one-half of a gravid segment from *H. robertrauschi* that was removed from the strobila at time of collection. We estimated the number of eggs fed to each beetle by counting the number of eggs from one individual gravid segment. An attempt was then made to use an individual of *Peromyscus leucopus* (Rafinesque 1818) as an experimental definitive host.

Discriminant analysis of five species of *Hymenolepis* was performed. Measurements of *H. weldensis* Gardner and Schmidt 1988, *H. geomydis* Gardner and Schmidt 1988, *Hymenolepis tualatinensis* Gardner 1985, and *H. diminuta* (Rudolphi 1819) were obtained from original archival data maintained in the Manter Laboratory and analyzed first by Gardner and Schmidt (1988). Measurements of the cestodes from *Onychomys* are from the following description. All multivariate procedures were performed using the statistical package SAS 9.3. All measurements except for variable No. 3 (number of segments) were scaled to micrometers before analysis and levels of statistical significance

were set a-priori at $p \leq 0.05$. To determine levels of deviation from normality of mensural characters used in the analysis, levels of skewness and kurtosis were calculated using integrated functions in Microsoft Excel™. The distribution of some variables did not conform to normality, so the data were log transformed

(\log_{10}) and checked again for normality. Subsequent analyses were then performed on the log-transformed data. Canonical variates analysis was performed on 17 character variables from 34 individual cestodes representing the five species of *Hymenolepis* (listed above) occurring in rodents.

RESULTS

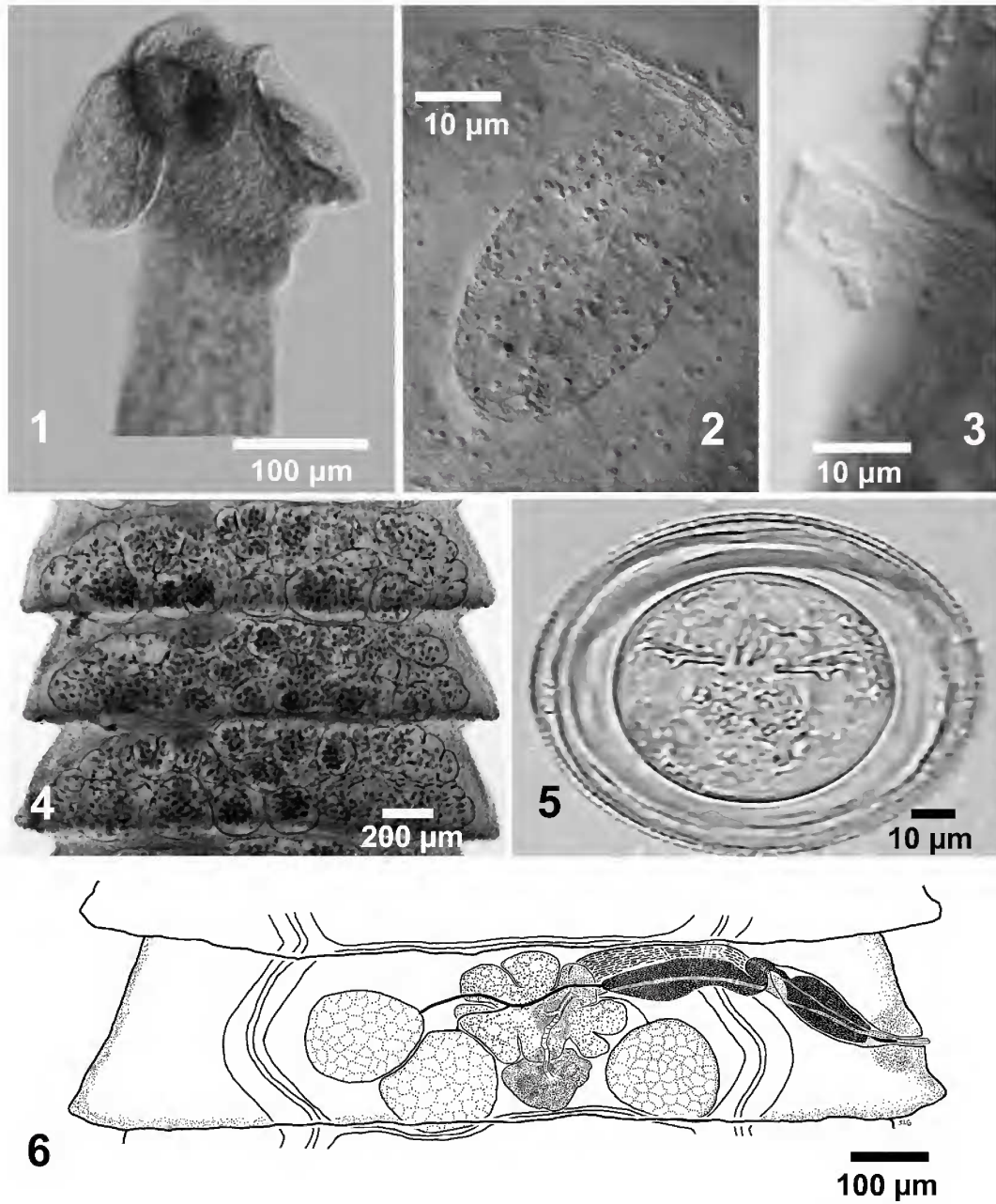
Following is a description of a new species of *Hymenolepis* s. str. Measurements are given in micrometers unless otherwise indicated. N is the number of individual structures of cestodes examined. In all specimens, measurements of each structure were averaged from measurements of five different segments whenever possible. Mean \pm SD are given in parentheses. Measurements of organs in mature segments were taken from the last mature segment, which was the one segment immediately anterior to the observed segment in which eggs begin to appear in the developing uterus. For measurements of egg characteristics, N represents the individual number of structures measured.

Hymenolepis robertrauschi n. sp.

Figs. 1-6

Description.—Eight full specimens were studied for the following description. Scolex (Fig. 1), N = 8, 199 - 257 (221 \pm 19) in maximum width. Suckers, N = 6, 119 - 164 (139 \pm 14) long by 82 - 95 (88 \pm 5) wide. Apical organ (AO) present; no evidence of osmoregulatory ducts associated with the AO (Fig 2.). AO with very small duct running antero-posterior starting at proximal end, not visible in Fig 2. Scolex unarmed. Neck, N = 8, 311-803 (572 \pm 211) long by 89-193 (143 \pm 33) in maximum width. Strobila, N = 8, 42.4 - 83.4 mm (63.0 \pm 13.9 mm) long, with 284 - 454 (343 \pm 67) segments; maximum width 1.18 - 2.54 mm (1.99 \pm 0.45 mm) attained late in gravid segments. Strobilar margins serrate with intersegmental boundaries well defined in mature and gravid segments. Segments (Fig. 6) wider than long; strobila attenuated anteriorly, with increase in relative length beginning in mature segments; length:width ratio of mature and gravid segments 0.20 - 0.29 (N = 8) and 0.20 - 0.30 (N = 7), respectively. Genital atrium, N = 9, 20 - 43 (34 \pm 8) deep. Genital pores unilateral, dextral. Genital ducts run dorsally across longitudinal excretory canals. Ventral canals, N = 8, 21 - 38 (26 \pm 6) wide, connected by

narrow transverse ducts. Dorsal canals, N = 9, 5 - 13 (8 \pm 3) wide. Cirrus sac elongate, fusiform, N = 8, 147 - 233 (193 \pm 32) in maximum length by 33 - 61 (47 \pm 8) in maximum width, with antiporal end usually barely overlapping excretory canals. Cirrus armed with minute spines (Fig. 3), approximately 1.3 long by 0.5 wide. Internal seminal vesicle, truncate, piriform, N = 9, 84 - 157 (128 \pm 27) in maximum length by 26 - 53 (44 \pm 8) in maximum width. External seminal vesicle, N = 7, 119 - 160 (138 \pm 13) long by 36 - 75 (54 \pm 12) in maximum width, anterior to poral testis. Testes subspherical, N = 9, 99 - 165 (131 \pm 20) long by 73 - 128 (105 \pm 17) wide, always with one poral and two antiporal, usually arranged with the antiporal testes slightly antero-posterior. Seminal receptacle, N = 7, 190 - 246 (216 \pm 19) long by 45 - 121 (84 \pm 27) in maximum width, extending porally, mostly anterior to ovary. Ovary lobate, globular, N = 7, 67 - 112 (86 \pm 19) in maximum length by 130 - 297 (192 \pm 62) in maximum width. Vitelline gland with relatively smooth margins, N = 9, 46 - 118 (83 \pm 23) wide by 45 - 102 (71 \pm 22) in maximum length, situated in posterior margin of segment near midline, ventral and posterior to ovary. In gravid segments (Fig. 4), uterus saccular, usually filling whole segment, rarely overlapping seminal receptacle, cirrus sac, and external seminal vesicle. Genital ducts always visible. Excretory canals passing through groove on ventral surface of uterus. Eggs, subspherical with thin outer shell (Fig. 5), N = 29, 57 - 76 (69 \pm 4) long by 44 - 58 (51 \pm 3) wide. Embryo oval (Fig. 5), N = 29, 40 - 57 (47 \pm 4) long by 31 - 53 (42 \pm 4) wide. Measurements of embryo hooks as follows: larger hooks of first and third pairs, N = 52, 15.7 - 20.5 (17.1 \pm 0.9) long by 3.1 - 4.9 (4.0 \pm 0.4) wide at guard; smaller hooks of first and third pairs, N = 42, 13.1 - 18.1 (16.2 \pm 1.1) long by 2.0 - 3.1 (2.6 \pm 0.3) wide at guard; middle pair of hooks identical in morphological characteristics, N = 32, 14.3 - 20.1 (17.3 \pm 1.2) long by 1.5 - 2.9 (2.2 \pm 0.3) wide at guard.



Figures 1-6. *Hymenolepis robertrauschi*. 1. Scolex. 2. Sac of apical organ showing no association of osmoregulatory ducts. 3. Cirrus with minute spines. 4. Gravid proglottids. 5. Egg. 6. Dorsal view of mature segment.

Symbiotype host.—Grasshopper mouse, *Onychomys leucogaster* (Wied-Neuwied 1841) (Rodentia: Cricetidae). Symbiotype Number: UNZC NP-1308.

Type locality/collection date.—16 km SW of Arthur, Arthur County, Nebraska, U.S.A.; lat. 41°28'55.9"N; long. 101°50'57.8"W; 30 July 2012.

Site of infection.—Small intestine, duodenum.

Prevalence.—82 of 372 specimens of *Onychomys* examined (22%)

Specimens deposited.—Holotype, HWML49792 (six gravid segments removed from holotype for life-cycle experiment, two segments stored in 10% formalin, 16 segments stored in 2% K₂Cr₂O₇ solution, ten segments stored in 98% ethanol and frozen at -80° C).

Specimens examined.—Paratypes: HWML49786, HWML49787, HWML49788, HWML49789, HWML49790, HWML49791; other specimens examined: HWML49795 - HWML49822.

Etymology.—This species was named after Dr. Robert L. Rausch who was one of the most influential helminthologists of the last two centuries and whose studies of mammals and helminth parasites has made a lasting influence on parasite systematics world-wide.

Life Cycle.—Cysticercoids of *H. robertrauschi* were discovered in the hemocoel of two individuals of *T. molitor* as early as 27 days post-infection. Only 33% of the beetles fed gravid proglottids became infected with cysticercoids of *H. robertrauschi*. In the two individuals of *T. molitor* that became infected, three cysticercoids were recovered from one and five from the other. The number of eggs per gravid proglottid of *H. robertrauschi* was estimated to be approximately 600; therefore, the rate of experimental infection of eggs of *H. robertrauschi* in *T. molitor* was approximately 0.4%. Gardner and Schmidt (1988) found a similar infection percentage for eggs of *H. weldensis* in *T. molitor*, while they showed that *H. diminuta* infected 100% of the beetles that were exposed with a much larger number of cysticercoids. The individual of *P. leucopus*, which was given three cysticercoids in saline using a pipette, did not become infected with *H. robertrauschi*.

Differential Diagnosis.—The genus *Hymenolepis sensu stricto* currently contains 13 species from rodents, four from bats, and one from hedgehogs (Makarikov and Tkach 2013). Because there is no evidence that species of *Onychomys* occurs anywhere but the Nearctic, we restrict our comparisons to those species occurring in rodents from the Nearctic region. Briefly, it is not possible that this species could occur anywhere where grasshopper mice do not live. In contrast, *H. diminuta* has a very large range in both geography and in mammals infected (occurring in all areas where *Rattus* has been reported). See justification in Gardner (1985) and Gardner and Schmidt (1988).

Hymenolepis robertrauschi can be recognized as distinct from *H. weldensis* based on the following easily discernible characters: shorter strobila, fewer segments, greater length:width ratio in mature and gravid segments, smaller ventral excretory canals, smaller seminal receptacle, and greater length of large hooks in embryo.

Hymenolepis robertrauschi can be recognized as distinct from *H. tualatinensis* by the following characters: smaller length:width ratio in gravid segments, genital ducts always dextral and never alternating, larger dorsal excretory canals, longer cirrus sac, longer internal and external seminal vesicles, and presence of three testes in all segments. Spines on cirrus of *H. robertrauschi* are larger and less dense than those of *H. tualatinensis* (the paratype examined was SLG71-4-A10).

Hymenolepis robertrauschi can be recognized as distinct from *H. geomydis* by the following characters: shorter strobila, fewer segments, greater length:width ratio in mature and gravid segments, smaller ventral excretory canals and larger dorsal excretory canals, longer cirrus sac, and presence of three testes in all segments. In addition, the life cycle was partially completed using *T. molitor* in *H. robertrauschi*, but never in *H. geomydis* (Gardner and Schmidt 1988).

Some published papers have provided evidence that *H. diminuta* and *Hymenolepis citelli* (McLeod 1933) are distinct, while some authors have not been able to show morphological differences in the strobilar stage (see summary in Gardner and Schmidt 1988). Therefore, the characters used to distinguish *H. robertrauschi*

from *H. diminuta* also will suffice to distinguish from *H. citelli*. *Hymenolepis robertrauschi* can be instantly separated from *H. diminuta* on the basis of the life cycle (see Gardner and Schmidt 1988).

Finally, *H. robertrauschi* can be distinguished from *Hymenolepis pitymi* Yarinsky 1952 by having a

larger strobila, much larger cirrus sac length, longer internal and external seminal vesicle lengths, larger ovary, much larger vitelline gland, and much larger eggs. Measurements of *H. pitymi* were taken from Gardner and Schmidt (1988) and Gardner (1985), who examined the type specimen.

DISCUSSION

Prevalence of Cestodes in Onychomys.—From 1989 through 1998 in New Mexico, 358 specimens of *Onychomys* were examined for helminths. This sample consisted of 153 individuals of *O. arenicola* and 205 individuals of *O. leucogaster*. *Hymenolepis robertrauschi* was found in 26% of the individuals of *O. arenicola* and 18% of the individuals of *O. leucogaster*, giving an overall prevalence of 22% in the individuals of *Onychomys* from New Mexico. The intensity of *H. robertrauschi* in *Onychomys* ranged from one to 33 individual cestodes. The number of individuals of *H. robertrauschi* found in each individual of *Onychomys* examined was generally between one and five; only seven of the 79 infected *Onychomys* harbored more than five individual cestodes.

In Nebraska, during August 2012 and July-August 2013, 14 specimens of *O. leucogaster* were examined for helminths; *H. robertrauschi* was found in four of these individuals.

Multivariate Analysis.—Seventeen character variables (Table 1) were analyzed that have been shown

to enable previous discrimination of four species (Gardner and Schmidt 1988). All measurements were taken from stained, mounted specimens. Characters of the scolex were not used for the multivariate analysis due to the small sample size of scolexes measured. Characters of the eggs were not included because of possible distortion of the egg shell and embryo by reagents and because it is usually necessary to use fresh material for analysis of these characters. The 17 characters chosen are easily identifiable and measurable in other groups of cestodes, and the analysis can be applied to morphologic measurements of preserved material. Therefore, the techniques may be applied to other groups with relative ease (see Gardner and Schmidt 1988).

The multivariate statistical analysis shows that all multivariate centroids are different for each species ($F = 8.86$, $df = 68, 53$) (see Fig. 7). Stepwise discriminant analysis (results not shown) indicated that the following characters were most important in separating among the species included in this analysis: number of segments, cirrus sac length, ovary width, cirrus sac width, seminal receptacle length, and internal seminal vesicle length.

Table 1. Character loadings and variation in canonical structure.

Variable	Can I	Can II	Can III
Maximum strobila length	-0.23	0.51	-0.27
Maximum strobila width	0.03	0.73	0.45
Number of segments	-0.15	0.85	-0.44
Cirrus sac length	0.72	0.56	0.004
Cirrus sac width	-0.15	0.32	0.61
Internal seminal vesicle length	0.72	0.58	-0.09
Internal seminal vesicle width	-0.01	0.24	0.66
External seminal vesicle length	0.43	0.64	0.17
External seminal vesicle width	0.35	0.66	0.28
Testes length	0.29	0.51	0.06
Testes width	0.05	0.66	0.14
Seminal receptacle length	0.29	0.75	0.06
Seminal receptacle width	0.29	0.59	0.15
Ovary length	-0.44	0.21	-0.25
Ovary width	-0.12	0.72	0.51
Vitelline gland width	-0.47	0.45	0.38
Vitelline gland length	-0.15	0.55	0.17
Percentage of variation of each canonical axis (CI)	51.53%	28.44%	17.13%
Summed variation accounted for by each CI	51.53%	79.97%	97.1%

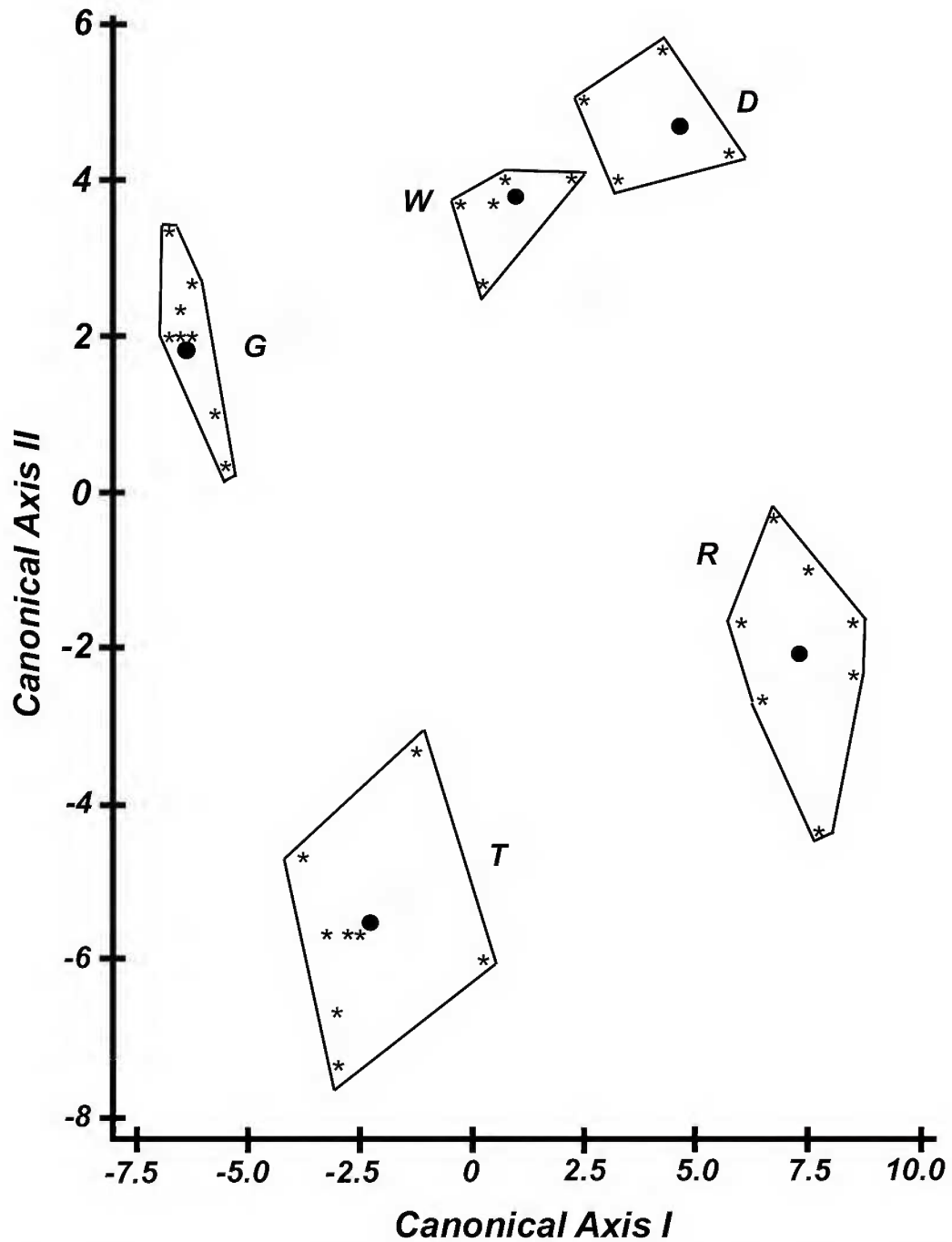


Figure 7. Ordination showing minimum polygons for each species from the first two canonical axes derived from morphometric analysis of *Hymenolepis*. Polygon labels as follows: G = *H. geomydis*; T = *H. tualatinensis*; W = *H. weldensis*; D = *H. diminuta*; R = *H. robertrauschi*. The centroid of each group is represented by a large black dot, and asterisks represent the scatter of individuals around each centroid.

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